REMARKS

Status of the Claims

Claims 1, 2, 8, 10-33 and 56 were presented for examination and were rejected. Claims 3-4 and 34-55 were withdrawn by the Examiner as being drawn to non-elected inventions. Claims 1 and 23 are herein amended to clarify the claims. No new matter has been added. Entry of the amendments and reconsideration in view of the following comments is respectfully requested.

With respect to all amendments, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Objection to the Specification

The specification was objected to because the trademark term "eppendorf" was not properly capitalized. The specification has been amended to address this concern. Accordingly, this objection may be withdrawn.

Withdrawn Rejections under 35 U.S.C. §§ 102 and 103

Applicants appreciate the Examiner's withdrawal of outstanding rejections under 35 U.S.C. §§ 102(b) and 103(a) in view of the previously submitted claim amendments.

New Rejection under 35 U.S.C. § 103

Claims 1, 2, 8, 10-33 and 56 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Fletcher *et al.* (*J. Gen. Microbiol.* 1976, 94:400-404, "Fletcher") in view of Kemshead & Ugelstad (*Mol. Cell. Biochem.* 1985, 67:11-18, "Kemshead") and Rudi *et al.* (*Appl. Environ. Microbiol.* 1998, 64:34-37, "Rudi").

The Office asserts that Fletcher teaches a method of recovery of marine bacteria from environmental samples using the well-known property inherent in polystyrene to non-specifically affix proteins and cells to the surface. Fletcher is cited solely to establish that it has long been known that untreated polystyrene *per se* is useful for non-specific biological sample concentration resulting in viable organisms. The Office acknowledges that Fletcher does not teach magnetic microbeads or any of the additional limitations of claims 2, 8, 10-33 and 56.

The Office further asserts that Kemshead teaches the use of magnetic materials for medical applications. The Office alleges that Kemshead teaches separation methods using magnetic microbeads for a variety of cell types, using both non-specific binding and specific binding partners. Thus, the Office concludes that the combination of Fletcher and Kemshead teaches that many types of cells can be separated and enriched from dilute environmental and clinical samples using magnetic polystyrene beads under a wide variety of conditions. Finally, Rudi allegedly teaches a method of using magnetic microbeads to sequentially separate bacteria from environmental samples and amplify separated DNA using the same magnetic microbeads.

The Office asserts that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Fletcher by substituting unmodified magnetic microbeads for polystyrene Petri dishes as taught by Kemshead and/or Rudi. The skilled artisan allegedly would have been motivated to do so because of the nonspecific adhesion of cells to polystyrene as taught by Fletcher and the rapidity of magnetic bead separation as taught by Kemshead and/or Rudi. The Office further alleges that there would have been a reasonable expectation of success, given well-known absorptive properties of polystyrene, as taught by Fletcher and the general utility of magnetic bead separation methods as taught by many researchers especially including Kemshead and/or Rudi. Therefore, the Office argues that the invention as a whole was clearly *prima facie* obvious to a person skilled in the art at the time of the invention.

Applicants respectfully traverse this rejection for the reasons set forth below.

The Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532 (Fed. Cir. 1993). Only if this burden is met does the burden of coming

forward with rebuttal argument or evidence shift to the applicant. *Id.* at 1532. When the references cited by the examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988). A *prima facie* case of obviousness requires the satisfaction of three requirements. First, the combined prior art references must teach or suggest all of the claim limitations. *In re Royka*, 490 F.2d 981, 985 (CCPA 1974); MPEP § 2143.03. Second, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1731 (2007). And third, there must be a reasonable expectation of success found in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991); MPEP § 2143.02.

There are a number of important differences between the teachings of Fletcher and the present invention. Fletcher teaches slow attachment of *Pseudomonas* bacteria to flat polystyrene Petri dishes over a period of 2 hours (page 400, second to last paragraph). As the Office correctly notes, Fletcher does not teach microbeads, magnetic or otherwise. Furthermore, Fletcher does not teach attachment of any cell types besides *Pseudomonas* to the polystyrene surface, particularly of any non-bacterial cells. Consequently, it is difficult to determine whether the attachment taught in Fletcher is non-specific, since non-specific or low specificity attachment should, by definition, be able to isolate a number of different cell types from a sample rather than one specific cell type.

In contrast, the present invention teaches isolation of different cell types (e.g., bacterial, epithelial, leukocytes; *see* Examples 1-3) by rapid (typical time 10 min, as elected in the Response to Restriction Requirement dated May 21, 2007; *see also* claim 22 and Examples 1-3), non-specific attachment of cells to small, magnetic microbeads (typical diameter 200 nm, as elected in the Response to Restriction Requirement dated May 21, 2007, page 2; *see also* claim 10 and Example 1). A typical mammalian cell is about 5-10 μm in diameter, and a typical bacterial cell is about 0.5-1 μm in diameter. This means that the magnetic microbeads of the present invention are typically smaller than the mammalian or bacterial cells binding to them. Consequently, the geometry of cell attachment to the magnetic microbeads of the present invention is completely different from the binding of *Pseudomonas* cells to a flat surface, where bacteria can form films by forming molecular connections with neighboring cells in addition to attaching to the plastic surface.

With respect to Kemshead, Applicants respectfully disagree with the Office's statement "Kemshead teaches separation methods using magnetic microbeads for a variety of cell types, using both non-specific binding and specific binding partners" (page 5 of the OA, citations omitted). Specifically he Office seems to take the position that Section II. Ways of generating magnetic cells of Kemshead (page 12) teaches magnetic cell separation by non-specific binding. This position is actually not supported by the cited portion of Kemshead, which is reproduced below in its entirety:

> **II. Ways of generating magnetic cells**
>
> *(A) Phagocytosis*
>
> If cells can be made to ingest magnetic compounds they may be simply removed from those incapable of rapid phagocytosis. Incubation of spleen cell suspensions with carbonyl iron, has become a classical procedure for depleting macrophages from cell suspensions (8). This results in a highly selective and simple depletion technique but is obviously of very limited application. Several trivalent lanthanide ions, e.g. erbium and dysprosium [*sic*], have been incorporated into cells as a way of rendering them magnetic (9). However whilst these ions are taken up by a variety of cells (WBC & RBC), rendering them 'magnetic', no selective uptake by specific cell types has been demonstrated. Use of these rare earth metals for manipulating cells in magnetic fields will therefore have to wait until either selective uptake mechanisms or targeting systems are developed.
>
> *(B) Pre-existing magnetic compounds*
>
> Whilst RBC are intrinsically non-magnetic the iron present in haemoglobin can be rendered paramagnetic in three different ways. Malarial parasites in red cells are known to cause the intracellular degradation of haemoglobin, to form breakdown products, some of which are magnetic (10) (See section VI). Removal of reversibly bound oxygen from haemoglobin also results in the generation of a paramagnetic iron/haem complex (11). This can be accomplished chemically by the addition of sodium dithionite to cells or physically by adaption of an artificial lung machine routinely used in surgical procedures (12). The efficiency of removal of dithionite treated RBC from suspension obviously depends on the effectiveness of generating paramagnetic haemoglobin and the choice of magnetic separation procedure. Two separate groups have shown that by using high

> gradient magnetic separation systems, 60-70% of dithionite treated RBC could be removed from solution, indicating the system was more useful for debulking cell populations, than for analytical separations (11, 13). The third method of rendering RBC paramagnetic is to form methemoglobin in erythrocytes. Treatment of oxyhaemoglobin RBC with sodium nitrite (15-30 mM) causes loss of one electron from each haem-iron to produce paramagnetic Met-Hb (14). This reaction obviously results in stable paramagnetic RBC that can be manipulated, and immunologically targeted to cells to render them magnetic.
>
> Because of the difficulties of generating paramagnetic RBC and the relative inefficiency of the procedures, magnetic separation techniques have not found favour in debulking RBC from whole blood. However the use of targeted paramagnetic RBC in cell separations has been explored in much more detail (See section IIIA).

Thus, Section II of Kemshead merely teaches different methods of rendering blood cells magnetic by introducing a magnetic substance inside the cells or by manipulating hemoglobin molecules inside red blood cells. There is no mention whatsoever of non-specific binding of cells to magnetic microparticles. A careful review of Kemshead further revealed that Kemshead only teaches targeting of magnetic particles to specific cell types using monoclonal antibodies, polyclonal antisera or other types of biomolecules (see pages 13-15). In fact, Kemshead teaches away from non-specific cell binding to magnetic microbeads by describing improved magnetic particle systems wherein non-specific cell binding was significantly reduced:

> Ugelstad and co-workers have continued to modify and improve these microspheres. Recently he has grafted a further hydrophobic polymer over the surface of the polystyrene beads increasing their size from 3 to 4 μm and reducing their surface area to approximately 5 m^2/g. This treatment has yielded microspheres completely monodisperse in suspension, that show almost no non-specific sticking to any human cell line examined (Ugelstad & Kemshead, unpublished).

(Kemshead at paragraph bridging pages 13 and 14, emphasis added).

> As can be seen above there are a host of different magnetic particles that can be used to couple to cells to render them magnetic. Using these microspheres impressive results have been obtained on

> magnetic cell separations in individual laboratories where expertise on specific systems has been built up. However no laboratory has screened the majority of microspheres to determine which are the best materials available. Particles used with hydrophobic polymers give far lower non-specific sticking to cells than hydrophilic [*sic*] microspheres. In addition homogeneity in the incorporation of paramagnetic materials either into microspheres or directly into carrier proteins affects the efficiency of the separation system. Microspheres of uniform size and magnetic content should theoretically improve any magnetic separation system. The microspheres developed by Ugelstad certainly fulfil [*sic*] the above criteria and can be used in conjunction with inexpensive samarium cobalt magnets.

(Kemshead at paragraph bridging pages 14 and 15, emphasis added).

Assuming, *arguendo*, that a combination of Fletcher and Kemshead teaches all the elements of claim 1, it is apparent that neither Fletcher nor Kemshead provides a motivation to combine the teachings of Fletcher and Kemshead to arrive at the presently claimed method with a reasonable expectation of success. As discussed above, Kemshead teaches that non-specific cell binding to magnetic microbeads is undesirable and should be reduced. Moreover, Fletcher teaches that binding of *Pseudomonas* bacteria to a flat polystyrene surface takes 2 hours and involves the formation of a bacterial film. Accordingly, it is unclear what would motivate a person skilled in the art at the time of the present invention to combine these teachings to arrive at the rapid, non-specific isolation of individual cells from a sample using magnetic microbeads of the present invention.

Rudi is cited for the proposition that the same magnetic microbeads may be used to sequentially separate bacteria from a sample and amplify bacterial DNA. As such, Rudi does not cure the deficiencies of Fletcher and Kemshead identified above.

Since a person skilled in the art would not have been motivated to combine the teachings of Fletcher and Kemshead and/or Rudi with a reasonable expectation of success, the Office has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully request that this rejection under 35 U.S.C. § 103(a) may properly be withdrawn.

Rejection under 35 U.S.C. § 112, ¶ 1, Enablement

Claims 1, 2, 8, 10-33 and 56 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for several of the recited magnetizable substances, allegedly does not reasonably provide enablement for those comprised of copper, tantalum or zirconium.

The Office asserts that copper, tantalum and zirconium are not known to exhibit magnetic behavior at normal temperatures. Accordingly, the Office argues that the specification and general knowledge in the art would allow a skilled artisan in the area of cell separation using magnetic microbeads to practice the method with known ferromagnetic and ferrimagnetic substances including magnetite, nickel and alloys of tantalum and zirconium, but not with the pure metals copper, tantalum and zirconium.

Without acquiescing to the Office's argument and solely to expedite prosecution of this matter, Applicants have amended claim 1 to address this concern. Accordingly, it is respectfully submitted that this enablement rejection under 35 U.S.C. § 112, ¶ 1 may properly be withdrawn.

Rejection under 35 U.S.C. § 112, ¶ 2

Claim 23 stands rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention because claim 23 contains the trademark/trade name "eppendorf".

The Office asserts that the claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product because a trademark or trade name is used to identify a source of goods, but does not identify or describe the goods associated with the trademark or trade name. In the present case, the Office takes the positions that the trademark "eppendorf" is used to identify a polymeric microcentrifuge tube and, accordingly, the identification is allegedly indefinite.

Applicants respectfully submit that the term "*eppendorf tube*" has become a generic term for *microfuge tubes* or *microcentrifuge tubes*. (*See*, *e.g.*, Wikipedia entry for "*Laboratory centrifuge*" at

http://en.wikipedia.org/wiki/Laboratory_centrifuge, attached as **Exhibit A**). Nevertheless, without acquiescing to the Office's argument and solely to expedite prosecution of this matter, claim 23 has been amended to recite "microcentrifuge tube" instead of "eppendorf tube". Support for the amendment may be found throughout the specification as published (Pub. No. US 2006/0141450 Al), for example, at paragraphs **[0010]** and **[0055]** and in FIG. 1. Accordingly, it is respectfully submitted that this indefiniteness rejection under 35 U.S.C. § 112, ¶ 2 may properly be withdrawn.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing **Docket No. 514572000600**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: November 11, 2008

Respectfully submitted,

Electronic signature: /Yan Leychkis/
Yan Leychkis
Registration No.: 60,440
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 314-7702